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Serine Synthesis in *Tetrahymena* from Non-amino Acid Sources; Compounds derived from Serine

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SUMMARY: Metabolic products of fermentable carbohydrates, as well as certain fatty acids, ethanol and acetaldehyde can serve as sources for serine synthesis in *Tetrahymena*. Exogenous hydroxypyruvate and related compounds were inactive.

Sparing experiments indicate that serine contributes to the formation of glycine, cysteine (presumably via cystathionine), aminoethanol and thymine.

It is becoming increasingly apparent that the serine-glycine interconversion usually represents a source of glycine to the organism rather than a source of serine. One of the most important precursors of serine appears to be carbohydrate or certain products of glycolysis (Arnstein, 1954, 1955; Black, Kleiber & Baxter, 1955; Ichihara & Greenberg, 1955, 1957; Kit, 1955; Nyc & Zabin, 1955; Sky-Peck, Pearson & Visser, 1956). Proposed routes are from 3-phosphoglycerate via hydroxypyruvate (Ichihara & Greenberg, 1955, 1957) or from hydroxyaspartate (Sallach 1955, 1956; Sallach & Peterson, 1956). Both pyruvate and acetate may serve as precursors of serine, and there are indications that a symmetrical intermediate may be involved in the synthesis from these precursors (Nyc & Zabin, 1955). In addition to acetate other fatty acids may be converted to serine (Black, Kleiber & Smith, 1952; Black, Kleiber, Smith & Stewart, 1957; Black & Kleiber, 1958).

In *Tetrahymena* also, it appears that, in addition to the ability to convert glycine to serine under the influence of high concentrations of folic acid (Dewey & Kidder, 1959), there is the possibility of utilizing carbohydrate and certain other substances for the synthesis of serine. Growth of the ciliate in the absence of serine appears to be rather susceptible to inhibition by carbohydrates as well as other possible intermediates, which makes interpretation of results difficult. Evidence has been obtained that glucose (or other fermentable carbohydrates), pyruvate, lactate, certain fatty acids, ethanol and acetaldehyde are precursors of serine for the growth of *Tetrahymena*. The dicarboxylic acids of the Krebs cycle, acetate and glycolate, have a small effect. Glycerate, 3-phosphoglycerate, dihydroxyacetone, glyceraldehyde, glyoxalate, hydroxypyruvate, dihydroxyfumarate, gluconate, tartrate and hydroxyaspartate either have no effect or are inhibitory.

Although serine can be synthesized from various precursors by all except one of the strains of *Tetrahymena* tested (Dewey & Kidder, 1960), media can be devised in which serine is a requirement for all strains. In such a medium it is possible to test for the activity of compounds which may be derived metabolically from serine. Compounds such as glycine, cysteine,

aminoethanol and thymine can be shown to spare the serine requirement of certain strains of *Tetrahymena* and in some cases appear to replace serine entirely.

METHODS

The cultural conditions and medium used were as previously described (Dewey & Kidder, 1960). Most of the data were obtained using *Tetrahymena pyriformis* strain W except as noted below. All media contained DL-threonine (88 $\mu\text{g./ml.}$), folic acid (0.01 $\mu\text{g./ml.}$) and thioctic acid (0.004 $\mu\text{g./ml.}$). Under these conditions appreciable serine synthesis from glycine does not occur. To this basal medium were added folic acid, thioctic acid, glucose (or other carbohydrates as noted), acetate, pyruvate, alanine, ethanol, acetaldehyde, glycolate, gluconate, glyoxalate (synthesized according to the method of Radin & Metzler, 1955), dihydroxyacetone, glyceraldehyde, phosphoglycerate, hydroxypyruvate (synthesized according to the method of Sprinson & Chargaff, 1946 or of Dickens & Williamson, 1958), hydroxyaspartic acid (obtained through the generosity of Dr W. Shive), dihydroxyfumarate, D-, L-, or meso-tartrate, fatty acids, Krebs cycle acids, glycine, cysteine, aminoethanol, thymine or lactate singly or in combination as indicated. All the above compounds were commercial samples except as noted. In most cases these compounds were added to the medium to test for sparing action in a dose response to serine or to glycine in the absence of serine. Unstable materials were sterilized by filtration through sintered glass. The data represent the averages of large numbers of experiments.

RESULTS AND DISCUSSION

A. Effect of compounds which may act as precursors of serine

As may be seen from Fig. 1, curve 1, there is some growth on glycine even at the level of folic acid ordinarily used in the medium, but that the addition of larger amounts of folic acid (0.1 $\mu\text{g./ml.}$) makes better growth possible (curve 3). This is a matter of the rate of growth, which is increased by the addition of folic acid (Dewey & Kidder, 1960). When glucose (2.5 mg./ml.) is added to the medium without increasing the folic acid content (curve 2), utilization of glycine is almost entirely suppressed. On the other hand, if both glucose and folic acid are added (curve 4) slight inhibition is apparent, by comparison with curve 3, only at the lower concentrations of glycine. Above a glycine concentration of 20 $\mu\text{g./ml.}$ glucose exerts a large stimulatory effect. While it is true that some of this stimulation must be due to the addition of an energy source, the effect is much greater than that obtained upon the addition of glucose to a medium containing serine (Fig. 2). Here glucose is seen to inhibit the utilization of suboptimal amounts of serine. With certain strains of *Tetrahymena* (for example strain E) glucose is even more inhibitory to the utilization of glycine than with strain W, but not to the utilization of serine. As the growth curves show (Fig. 3), at the usual time of measurement of turbidity (96 hr.) strain E would be considered to be unable to utilize glycine in the

Fig. 1. Dose response of *Tetrahymena pyriformis* W to glycine. Curve 1, basal medium; curve 2, with glucose, 2.5 mg./ml.; curve 3, with folic acid 0.1 μg./ml.; curve 4, both present.

Fig. 2. Dose response of *Tetrahymena pyriformis* W to serine with (curve 1) and without (curve 2) glucose (2.5 mg./ml.).

presence of glucose. Glycerol as well as all of the mono and disaccharides which are utilized by the ciliate (glucose, fructose, mannose and maltose) have a similar stimulatory effect. Those sugars which are not utilized (galactose and lactose) are inhibitory (Table 1).

Table 1. *Effect of a representative fermentable (glucose) and non-fermentable (lactose) carbohydrate on serine synthesis in Tetrahymena pyriformis W in the presence and absence of glycine*

		Glucose (mg./ml.)					
		0	0.10	0.25	0.50	1.00	2.50
		Optical density					
Minus glycine		0.22	0.33	0.30	0.23	0.26	0.19
Plus glycine (90 µg./ml.)		0.36	0.48	0.47	0.41	0.49	0.49
		Lactose (mg./ml.)					
		0	0.10	0.25	0.50	1.00	2.50
		Optical density					
Minus glycine		—	—	0.27	0.26	0.24	0.21
Plus glycine (90 µg./ml.)		—	—	0.36	0.34	0.32	0.27

Medium contains acetate (1 mg./ml.); folic acid (0.1 µg./ml.).

Of the glycolytic products of glucose: dihydroxyacetone, glyceraldehyde and 3-phosphoglycerate, all were inhibitory while glycerate had little or no effect. Hydroxypyruvate, which is derived metabolically from phosphoglycerate (Ichihara & Greenberg, 1955, 1957), was inhibitory (first preparation, which probably contained some bromopyruvate) or had no effect (second preparation) either with or without the addition of alanine (Sallach, 1955). On the other hand, pyruvate or lactate had the same stimulatory effect as glucose, although the addition of larger amounts of thioctic acid (0.04 µg./ml.) is required if lactate is to be fully effective (Fig. 4). Thioctic acid is also stimulatory when either glucose or pyruvate is present in the medium, but the effect is not as great as in the case of lactate. Increased growth in the presence of either glycine or of additional folic acid as well as pyruvate may be explained either by the provision of an additional source of serine or the removal of the necessity for the synthesis of glycine from the serine produced. Thioctic acid and folic acid are the only vitamins which can be shown to affect serine synthesis in *Tetrahymena pyriformis* W. Pyridoxal (Blakley, 1955) was without specific effect.

Since it is advantageous to add alanine to media containing hydroxypyruvate in order to be certain that transamination to serine is possible (Sallach, 1955), it was necessary to study the effect of alanine alone on serine synthesis. As may be seen from Figs. 5 and 6, alanine increases growth in the absence of serine whether or not glycine is present. This effect is, however, much smaller than that exhibited by pyruvate. It is possible that there may

be some conversion of alanine to pyruvate and eventually to serine. The fact that alanine is much more effective when other precursors of serine are present suggests, however, that it may be acting as an amino donor. There is also an additional stimulatory effect of alanine even in the presence of adequate serine. It appears that the organism is relatively deficient in a system for the biosynthesis of alanine. It has also been shown (Dewey & Kidder, 1958) that alanine may act as a competitive analogue of serine. The amounts of alanine required to show this inhibition are, however, relatively large (for strain W) and it is quite possible that endogenously produced serine is less susceptible to this type of inhibition than that supplied exogenously.

Fig. 3. Growth curves of *Tetrahymena pyriformis* E. Curve 1, serine; curve 2, glycine; curve 3, serine + glucose; curve 4, glycine + glucose. Serine or glycine 250 $\mu\text{g./ml.}$; glucose 2.5 mg./ml.

Since acetate is a component of the medium generally used for the cultivation of *Tetrahymena* and because it is produced metabolically from pyruvate, the effect on serine synthesis of the addition of acetate to the basal medium was tested. As may be seen (Fig. 4) acetate had only a slight stimulatory effect as compared to pyruvate. The further addition of thioctic acid had little effect. It appears that the function of thioctic acid in serine synthesis is not primarily in the oxidation of pyruvate to acetate, but rather in the oxidation of lactate to pyruvate (Kline, Pine, Gunsalus & Barker, 1952), where high levels may be required.

Other two-carbon compounds were also tested for their effect on serine

synthesis. Of these ethanol (1–4 mg./ml.) was found to show an appreciable stimulation. This was discovered when the thioctic acid level of the medium was raised. At that time the thioctic acid stock solution was prepared in ethanol. When thioctic acid was found to stimulate serine synthesis, it was re-tested in aqueous solution and the effect of ethanol was tested separately. It was found that both are stimulatory and that their effects are additive. In view of the small effects produced by acetate, it seems improbable that ethanol is oxidized to acetate before utilization.

Fig. 4. Dose response of *Tetrahymena pyriformis* W to glycine. Curve 1, basal medium; curve 2, lactate 250 $\mu\text{g./ml.}$; curve 3, lactate + thioctic acid 0.04 $\mu\text{g./ml.}$; curve 4, pyruvate 250 $\mu\text{g./ml.}$; curve 5, pyruvate + thioctic acid; curve 6, acetate 1 mg./ml.

Acetaldehyde was also tested as a possible intermediate in ethanol utilization. Only relatively small concentrations of the aldehyde can be used (10–50 $\mu\text{g./ml.}$), however, because of inhibitory effects. Nevertheless the activity of these low concentrations of acetaldehyde indicate that it is quantitatively more active than either ethanol or acetate. Acetate is required, however, if either acetaldehyde or ethanol is to show maximum activity. This suggests that a $\text{C}_2\text{--C}_2$ condensation takes place between two dissimilar moieties, e.g. acetate–acetaldehyde, acetate–ethanol or acetaldehyde–ethanol, although it is also possible that the ethanol is first oxidized to acetaldehyde or contains a slight contamination of aldehyde. In any case the Thunberg–Wieland condensation of acetate to produce succinate, although it is known to occur

in the ciliate (Barron & Ghiretti, 1958), or the condensation of ethanol to give succinate or fumarate (Foster *et al.* 1949), must be less effective.

In addition to acetate other fatty acids were also tested for their effect on serine synthesis. Of these propionate and other fatty acids containing an odd number of carbon atoms (which might serve as precursors of propionate) proved to be particularly effective. In the form of their coenzyme A derivatives, acrylic acid, β -hydroxypropionic acid, β -alanine and lactic acid have been found to be products of propionate metabolism (Vagelos, Earl & Stadtman, 1959). Of these only lactate is formed from acrylate by animal tissue and

Fig. 5. Comparison of effects of alanine and pyruvate on dose response of *Tetrahymena pyriformis* W to glycine. Curve 1, basal medium; curve 2, DL-alanine 200 $\mu\text{g./ml.}$; curve 3, pyruvate 250 $\mu\text{g./ml.}$

is the only one of the group which stimulates growth of *Tetrahymena* in the absence of serine. Probably the predominant route of propionate metabolism, however, is its conversion to succinate by fixation of CO_2 (Flavin, 1955; Lardy & Adler, 1956; Katz & Chaikoff, 1955; Wolfe, 1955; Flavin, Castro-Mendoza & Beck, 1956; Leaver & Stjernholm, 1956; Landau, Ashmore, Zottu & Hastings, 1959). *Tetrahymena* also fixes CO_2 with succinate formation (Van Niel, Rubin, Carson, Kamen & Foster, 1942). Evidence has been presented that a symmetrical four-carbon intermediate appears to be involved in the conversion of propionate to serine in the dairy cow (Black, Kleiber & Smith, 1952; Black & Kleiber, 1955, 1958). A symmetrical intermediate is

also required to explain the isotope distribution in serine when either acetate or pyruvate is used as a precursor (Nyc & Zabin, 1955).

While it is generally accepted that cells are poorly permeable to dicarboxylic acids, a number of Krebs cycle acids was tested for effects on serine synthesis and found to have only slight activity. Of the other symmetrical four-carbon compounds available, dihydroxyfumaric acid and the various isomers of tartaric acid were inactive, although they are presumably derived from Krebs cycle intermediates (Kun & Hernandez, 1955; Kun & Davis, 1956). Since hydroxyaspartic acid is also produced from these intermediates (Sallach, 1956; Sallach & Peterson, 1956) its effect on serine synthesis was tested. This

Fig. 6. Dose response of *Tetrahymena pyriformis* W to glycine; effect of alanine and precursors of serine. Curve 1, acetate 1 mg./ml.; curve 2, acetate + DL-alanine 200 µg./ml.; curve 3, acetate + glucose 2.5 mg./ml.; curve 4, acetate + glucose + alanine.

compound proved to be rather inhibitory to growth, but this may be due to the fact that an unresolved mixture of the four possible isomers was used.

One other possible source of symmetrical four-carbon acids is the glyoxylate cycle of Kornberg & Beevers (1957), by which acetate is condensed with glyoxylate to produce malate. Since glyoxylate has no effect on serine synthesis either this cycle does not operate in *Tetrahymena* or malate is not on the route to serine.

Although a number of compounds which should lead to the synthesis of serine via hydroxypyruvate, including hydroxypyruvate itself, are inactive in replacing serine for the growth of *Tetrahymena*, certain others which must lead to the formation of these compounds metabolically are active. One is

Fig. 7

forced to rely for explanation of such results on the possibility that cells are impermeable to certain compounds or the probability that these compounds are utilized only in an activated form. In the latter case the organism must lack specific kinases for those compounds which must be utilized as a phosphorylated derivative, or specific enzymes for the formation of the acyl coenzyme A intermediates. Figure 7 illustrates the above possibilities in which dotted lines denote apparently inoperative pathways in *Tetrahymena*.

B. *Compounds for which serine may be a precursor*

Aside from incorporation into protein, serine is used for the production of a number of other metabolically important compounds such as glycine (Arnstein, 1954), aminoethanol (Levine & Tarver, 1950) (used in the synthesis of choline, Kidder & Dewey, 1951), cysteine (Genghof, 1949, 1951) and C₁ fragments (Elwyn & Sprinson, 1954; Dewey & Kidder, 1953; Heinrich, Dewey & Kidder, 1957). These relationships are shown in Fig. 8. Strains GHH, E and W were chosen for comparison of the sparing effects of serine derivatives on growth.

Fig. 8

Strain GHH does not synthesize serine under any conditions so far devised, strain E requires large amounts of folic acid when glycine is supplied as the serine source while strain W is very active in serine synthesis (Dewey & Kidder, 1960). Table 2 shows that the addition of any one or various combinations of compounds has no effect on the growth of strain GHH in the absence of serine. Either cysteine or aminoethanol improves growth at low levels of serine, while acetate + glucose or alanine give improvement only at higher levels of serine.

Table 2. *Response of Tetrahymena pyriformis GHH to compounds sparing serine*

Additions	DL-serine (μg./ml.)						
	—	10	20	30	40	50	250
Optical density							
None	0.02	0.04	0.09	0.16	0.20	0.23	0.32
Glycine (20 μg./ml.)	0.02	0.04	0.06	0.15	0.21	0.24	0.32
Cysteine (20 μg./ml.)	0.02	0.08	0.14	0.18	0.21	0.23	0.36
Aminoethanol (20 μg./ml.)	0.02	0.10	0.15	0.18	0.21	0.23	0.35
Acetate + glucose	0.02	0.04	0.10	1.18	0.25	0.29	0.45
All + DL-Alanine (110 μg./ml.)	0.01	0.09	0.22	0.29	0.33	0.39	0.54

Na acetate, 1 mg./ml.; glucose 2.5 mg./ml., where present.

The former response is typical of a sparing effect, the latter of non-specific stimulation.

Table 3 illustrates similar experiments performed with strain W. Sparing effects are obtained upon the addition of small amounts of either glycine, cysteine or aminoethanol singly to the medium. These results are very similar to those obtained with strain GHH. Even in the absence of serine growth is obtained, however, when all of the compounds capable of sparing serine are present at the same time along with an adequate amount of alanine. This is true whether or not acetate and glucose are present and probably represents sparing to the point where only minimal amounts of serine need be synthesized. It is to be noted that thymine shows a small sparing effect with strain W.

Table 3. *Response of Tetrahymena pyriformis W to compounds sparing serine*

Additions	DL-serine ($\mu\text{g./ml.}$)						
	0	5	10	15	20	25	250
	Optical density						
None	0.04	0.08	0.13	0.17	0.19	0.22	0.31
Glycine (20 $\mu\text{g./ml.}$)	0.08	0.08	0.14	0.16	0.18	0.21	0.38
Cysteine (20 $\mu\text{g./ml.}$)	0.08	0.08	0.14	0.19	0.21	0.24	0.16
Aminoethanol (20 $\mu\text{g./ml.}$)	0.05	0.14	0.17	0.20	0.22	0.22	0.32
All	0.41	0.45	0.45	0.45	—	0.41	0.41
All + thymine (20 $\mu\text{g./ml.}$)	0.44	0.46	0.48	0.47	0.47	0.47	0.44
All + thymine + alanine	0.48	0.50	0.51	0.50	0.50	0.48	0.45
Acetate + glucose	0.04	0.12	0.18	0.22	0.26	0.29	0.47
All of above	0.52	0.57	0.60	0.60	0.61	0.64	0.68

Na acetate, 1 mg./ml.; glucose, 2.5 mg./ml.; DL-alanine, 110 $\mu\text{g./ml.}$, where present.

The serine requirement of strain E is spared to the greatest extent by cysteine, while glycine and aminoethanol have smaller effects. Either alanine or thymine appear to be inhibitory alone, but in combination with the other compounds they have a growth-promoting effect (Table 4). Growth is still slow (7 days) in the absence of serine even when the requirement is spared by the addition of all of the active compounds together (Elliott & Hogg, 1952; Dewey & Kidder, 1960). These facts may explain the earlier reports that strain E has an absolute requirement for serine (Elliott, 1949, 1950).

A comparison was made of the available strains with regard to the effects of alanine and of compounds sparing serine. In most cases alanine is inhibitory at low levels of serine, but may show stimulation in the presence of adequate amounts of serine. With a few exceptions aminoethanol appears to have the largest sparing effect. As may be seen from table 5, the addition of all of the active compounds permits excellent growth in the absence of serine of all of the strains except GHH and S.

The data presented above indicate that *Tetrahymena* also carries out most of the reactions in Fig. 8. The ability of the ciliate to convert serine to sphingosine (Brady & Koval, 1958) has not been tested. Differences in the

sparing activity of the various compounds from strain to strain may reflect differences in the levels of the enzymes implicated in the reactions. All strains, for example, appear to have a relative deficiency in serine decarboxylase, since aminoethanol has a pronounced sparing effect. This may, however, indicate only that the quantitative requirement for aminoethanol is larger than that for other compounds.

Table 4. *Response of Tetrahymena pyriformis E to compounds sparing serine (7 days growth)*

Additions	DL-serine ($\mu\text{g./ml.}$)					
	0	5	10	15	20	250
	Optical density					
None	0.08	0.04	0.10	0.18	0.17	0.25
Glycine (20 $\mu\text{g./ml.}$)	0.04	0.04	0.11	0.20	0.17	0.26
Cysteine (20 $\mu\text{g./ml.}$)	0.13	0.17	0.16	0.16	0.16	0.26
Aminoethanol (20 $\mu\text{g./ml.}$)	0.11	0.18	0.15	0.16	0.16	0.26
All above	0.10	0.15	0.17	0.18	0.19	0.26
DL-Alanine (20 $\mu\text{g./ml.}$)	0.02	0.03	0.06	0.14	0.17	0.24
Thymine (20 $\mu\text{g./ml.}$)	0.02	0.03	0.08	0.15	0.15	0.23
All above	0.18	0.20	0.18	0.16	0.17	0.25
Acetate + glucose	0.03	0.15	0.12	0.22	0.26	0.39
All above	0.24	0.30	0.30	0.30	0.29	0.38

Na acetate, 1 mg./ml.; glucose, 2.5 mg./ml., where present.

Table 5. *Growth response of various strains of Tetrahymena in the presence or absence of serine*

Additions	Strain			
	W*	E†	GHH	S
	Optical density			
None	0.02	0.02	0.01	0.02
Serine	0.37	0.21	0.33	0.18
Gly, Cys, Thy, Ame	0.42	0.19	0.02	0.05
+ Alanine	0.46	0.17	0.02	0.04
+ Glucose	0.56	0.24	0.02	0.04
+ Ace, Glucose	0.52	0.25	0.02	0.05

* Representative also of strains T, GC, H, GL.

† Representative also of strains GP, Gl-R, Ch-S, WH6, CM, V, PP.

Serine = DL-serine, 250 $\mu\text{g./ml.}$; Alanine = DL-alanine, 110 $\mu\text{g./ml.}$; Gly = glycine, 20 $\mu\text{g./ml.}$; Cys = L-cysteine, 20 $\mu\text{g./ml.}$; Thy = thymine, 20 $\mu\text{g./ml.}$; Ame = aminoethanol, 20 $\mu\text{g./ml.}$; Ace = Na acetate, 1 mg./ml.; Glucose at 2.5 mg./ml.

It would appear that in supplying glycine, cysteine, aminoethanol (choline) and thymine the major products of serine metabolism are made available to the ciliate so that the relatively small amounts of serine required as such for the synthesis of protein and phospholipids can be synthesized by the ciliates (except strain GHH and possibly S) from such compounds as glucose.

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